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No Evidence for the Compensation Hypothesis in the Swelled Vent Frog (*Feirana quadranus*)

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Abstract The compensation hypothesis predicts that if the left testis is defective e.g. due to developmental stress, the increased right testis serves a compensatory role, and thereby displaying testes asymmetry which can be a reliable indicator of male body condition. Here, to test the prediction of the compensation hypothesis, we analyzed difference in size between left testis and right testis and the relationship between testes asymmetry and male body condition in the swelled vent frog (*Feirana quadranus*). We found that the left testis was larger than right testis, displaying a significant directional asymmetry in testes size. Although testes mass was correlated with body condition, testes asymmetry was not correlated with body condition, which cannot provide evidence that the right testis had a compensatory function. Our findings suggest no evidence for the compensation hypothesis in this species due to lacking the compensatory function in right testis.

Keywords compensation hypothesis, body condition, *Feirana quadranus*, testes asymmetry, testes mass

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1. Introduction

As one of energetically expensive organs in organisms, testis size plays an important role in mediating pre- and postmating sexual traits during the breeding process (Liao *et al.*, 2018; Zhong *et al.*, 2018; McCullough *et al.*, 2018; Joseph *et al.*, 2018; Cai *et al.*, 2019). Testis size exists variations within or among species which are associated with mating system and habitat types (Liao et al., 2013a,b; Zeng et al., 2014; Chen *et al.*, 2016; Jin *et al.*, 2016a, b; Tang *et al.*, 2018). The compensation hypothesis states that one testis may compensate for a reduced function in the other testis by growing more (Lake, 1981; Møller, 1989; Jamieson et al., 2007). There are evidences that significant difference in testis mass between left and right is commonly observed in animal kingdoms (birds: Birkhead *et al.*, 1997; anurans: Hettyey *et al.*, 2005; Zhou *et al.*, 2011; Zhou *et al.*, 2011; Mi *et al.*, 2012; fishes: Liu *et al.*, 2014). Hence, if the left testis becomes nonfunctional e.g. due to developmental stress, the right testis should increase in size (Lake, 1981; Birkhead *et al.*, 1997; Graves, 2004).

Previous studies been shown that the degree of testes asymmetry varies with body condition within species (Møller, 1994; Hettyey *et al.*, 2005; Liu *et al.*, 2011). For instance, males with poor body condition have more symmetric testes than males with strong body condition in common frog (*Rana temporaria*) based on the sensitivity of developmental stress (Hettyey *et al.*, 2005). Meanwhile, body size in male *R. nigromaculata* is negatively correlated with the degree of testes asymmetry, which is consistent with the prediction of the compensation hypothesis that the right testis displays a compensatory role when the left testis is malfunctioning (Zhou *et al.*, 2011). However, testes asymmetry in some species does not follow

prediction of the compensation hypothesis (Liu *et al*., 2011; Liu *et al*., 2014). Hence, it is still unclear whether the compensation hypothesis would be a general model for testes asymmetry, or it is a more specific hypothesis that only applies to some taxa.

The swelled-vent frog (*Feirana quadranus*) inhabits montane streams in the Qinling–Daba Mountains at elevations ranging from 335 to 1830 m (Zhong *et al*., 2018). In this species, egg-laying extends from early April to mid-May, and this species is classified as a prolonged breeder (Wells, 1977). Although phylogeography and altitudinal variations in relative testes size and gut length have been addressed in the Qinling–Daba Mountains in recent years (Wang *et al*., 2012; Tang *et al*., 2018; Mai *et al*., 2019), testes asymmetry in this specie is unavailable. Here, our aims were to investigate testes asymmetry and to test the compensation hypothesis in the swelled vent frog (*F. quadranus*). We first analyzed whether individuals possess displayed significant (directional) differences in mass between in left testis and right testis mass. We consequently tested the prediction of the compensation hypothesis whether the positive correlation exists between degree of testis asymmetry and male body condition.

2. Materials and Methods

A total of 66 males were collected from three populations in breeding season along an altitudinal gradient at Qinling–Daba Mountains in western China between May and July in 2017 (Table 1). We captured all individuals in streams by hand at night with a 12-V flashlight (Wang *et al*., 2020). All individuals were brought back to the laboratory and kept at room temperature in an individual rectangular tank (1 m × 0.5 m × 0.8 m, L × W × H) with freshwater of 2 cm depth. We did not give them food before killed. Each individual was killed using single-pithing (Mai *et al*., 2017a,b). Body size (snout-vent length: SVL) of each individual was measured to the nearest 0.1mm using a vernier caliper and body mass to the nearest 0.1mg using an electronic balance. We took out two testes and weighed them to the nearest 0.1mg using an electronic balance. All dissections and measurements were performed by one person (Tang T). Finally, all specimens were preserved in 4% phosphate buffered formalin (Liu *et al*., 2018). Following the proposal of Møller and Swaddle (1997), the degree of directional testes asymmetry was used by the absolute and relative testes asymmetry. The absolute testes asymmetry = left testis mass – right testis mass. Relative testes asymmetry = left testis mass – right testis mass / 0.5 (left testis + right testis mass).

All statistical analyses were performed using SPSS22.0 (Statistical Product and Service Solutions Company, Chicago, USA). Body size, body mass and testes mass were log-transformed to achieve normality and improve homogeneity of variances. To test for differences in body mass, SVL and testes mass among populations, we treated body mass, SVL, left and right testes mass as dependent variables, population as a main factor using one-way ANOVA. We used paired *t*-test to test for differences in mass between left and right testis.

To avoid collinearity between body mass and SVL, we used body condition (= $\log_{10}$ (body mass) being divided by $\log_{10}$ (SVL)) as one variable in following analysis (Mi *et al*., 2012). We used Pearson correlation analysis to test the relationships between absolute testes asymmetry and male body condition, and between relative testes asymmetry and male body condition.

3. Results

Body mass, SVL, left testis mass and right testis mass did not differ among populations (Table 1; one-way ANOVA: all $P > 0.05$). There was significant difference in size between left testis and right testis, with left testis being larger than right one (Figure 1; paired *t*-test, $t = 5.395$, $n = 63$, $P < 0.001$). The left testis mass tended to be positively correlated with the degree of relative testes asymmetry (Pearson correlation analysis: $r = 0.195$, $n = 64$, $P = 0.123$).

Male body condition was positively correlated with testes mass (Figure 2; Pearson correlation analysis: $r = 0.348$, $n = 64$, $P = 0.005$). Male body condition was not correlated with degree of absolute testes asymmetry ($r = -0.022$, $n = 64$, $P = 0.861$) and degree of relative testes asymmetry ($r = -0.093$, $n = 64$, $P = 0.467$).

4. Discussion

Our results reveal that left testis mass significantly are larger than right testis mass among 64 individuals, and thereby displaying a directional testis asymmetry. Also body condition is positively correlated with testes mass. We do not find a positive

Table 1 Location, sampling, means and standard deviations of body size, somatic mass and testes mass among *Feirana quadranus* populations in Qinling-Daba Mountains.

Sites	Latitude	Longitude	Sampling	SVL (mm)	Body mass (g)	Left testes mass (mg)	Right testes mass (mg)
835-m site	32.59°	106.56°	36	80.7 ± 8.6	48.2 ± 13.0	336.9 ± 243.3	313.8 ± 361.1
977-m site	32.62°	106.47°	10	79.9 ± 5.6	40.6 ± 8.3	216.5 ± 138.2	194.8 ± 94.2
1289-m site	32.73°	106.87°	18	74.8 ± 5.7	46.5 ± 13.1	589.3 ± 453.0	539.7 ± 219.4

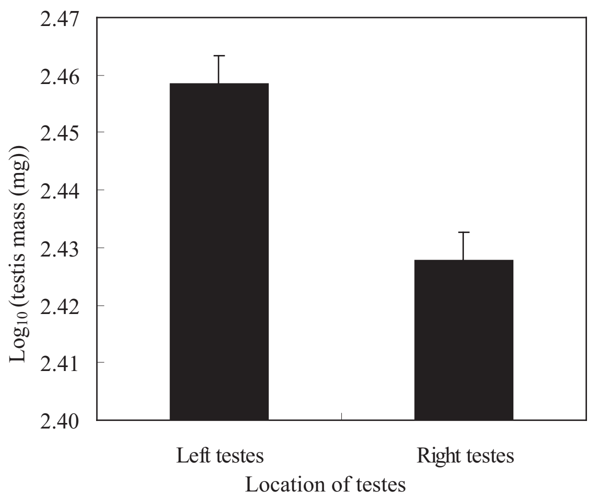

Figure 1 Difference between left testes and right testes mass in *Feirana quadranus* in western China.

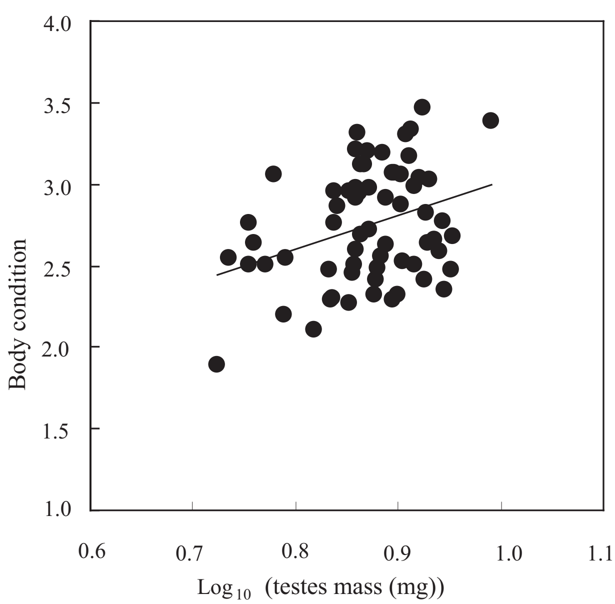

Figure 2 A positive correlation between testes mass and body condition in *Feirana quadranus* in western China.

relationship between degree of testes asymmetry and male body condition, which is inconsistent with Møller's prediction of the compensation hypothesis (Møller, 1994).

Previous studies have shown that the left testis size is larger than the right one in birds (Wright and Wright, 1944; Rising, 1987; Møller, 1994; Birkhead *et al.*, 1997; Yu, 1998) whereas the right testis is larger than the left one in some species (Friedmann, 1927; Ligon, 1997). In frogs, the left testis mass is also significantly larger than the right one in the Guenther's frog *Hylarana guentheri* (Liu *et al.*, 2011) and the dark-spotted frog *R. nigromaculata* (Zhou *et al.*, 2011). However, Hettyey *et al.* (2005) found that males from all populations have larger right testes than left testes in *R. temporaria*, as well as for *Carduelis chloris* (Merilä and Sheldon, 1999). If the left testis becomes nonfunctional (Lake, 1981; Møller, 1994; Birkhead *et al.*, 1997), the right testis should exhibit an increase in size, which can explain a larger right testis. The higher efficiency in sperm production of the left testis occurs in embryonic development (Kempenaers *et al.*, 2002). As a result, a larger left testis becomes advantageous when a smaller right testis display a compensatory function (Birkhead *et al.*, 1998). Hence, the left testis mass was expected to be positively correlated with degree of relative testes asymmetry. Indeed, this pattern has been previously observed in *H. guentheri* where the left testis size is significantly correlated with degree of relative testes asymmetry (Liu et al., 2011). In this study, left testis mass was not correlated with the degree of relative testes asymmetry in *F. quadranus*.

Game theory models state that the risk and intensity of sperm competition can promote an increase of ejaculate investments (Parker, 1998). In particular, when the intensity of sperm competition is high, the testes should become relatively large (Møller, 1991; Møller and Briskie, 1995; Liao *et al.*, 2011; Liao *et al.*, 2018). The evidence of heritability of body condition reveals that the purposes of sexual selection in females are to use the genetic association between sperm traits and body condition (Merilä *et al.*, 2001; Simmons and Kotiaho, 2002; Schulte-Hostedde and Millar, 2004). As a result, for males testes mass should be positively correlated with body condition (Simmons and Kotiaho, 2002). For most species of frogs, the relative testes mass is positively associated with body condition and age, suggesting that males with good body condition exhibit the higher chances of reproductive success (Hettyey *et al.*, 2005; Liao and Lu, 2009; Liao and Lu, 2011a,b; Mi *et al.*, 2012). Here, there was a positive correlation between relative testes mass and body condition, which suggested that males with good conditions improve their ability of sperm competition through the increased investments of testes.

The relationship between body condition and degree of testis asymmetry can be understood as adaptive, by causing physiological or morphological handicaps that males with good condition can deal with (Møller, 1994). For instance, the degree of directional testes asymmetry is positively correlated with body condition in the house sparrow and barn swallow (Møller, 1994) and the common frog (Hettyey *et al.*, 2005). Testis asymmetry represents the optimal phenotype which thus should be the norm. However, most studies have shown that testes asymmetry may not be a good measure of male body condition and display no relationship between them (Birkhead *et al.*, 1997; Birkhead *et al.*, 1998; Kimball *et al.*, 1997; Kempenaers *et*

al., 2002; Liu *et al*., 2014). Consistent with those of *R. nigromaculata* and *H. guentheri* where the degree of testes asymmetry is not correlated with male body condition (Liu *et al*., 2011; Zhou *et al*., 2011), we found that individuals with strong condition did not display higher degree of testes asymmetry than those with poor condition.

In conclusion, we found a directional testes asymmetry, with the left testis mass being larger than the right testis one. However, our results of the degree of testes asymmetry was not positively correlated with body condition, which were inconsistent with the prediction of the compensation hypothesis.

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